

Immunology , Mycology Department

e of food borne pathogen in some types of soft cheeses in The

**A Thesis Submitted to
Faculty of Veterinary Medicine
Bennha University**

VIA

Mohammed Mahmoud Mohammed Yousef Wehaba
(B.V.Sc., Kafer Elshiekh University, 2003) For the degree of M.V.Sc.
(Bacteriology, Immunology, Mycology)



**Under Supervision of
Prof.Dr. Ashraf Awad Abed El tawab**

**Professor of Bacteriology ,Immunology , Mycology Department
Faculty of Veterinary Medicine**

Benaha University

Prof.Dr. Amal Mostafa Eid

Chief Researchs

**Egyption Animals Health Researchs Institutes,
Tanta branch**



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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
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صَدَقَ اللَّهُ الْعَظِيمَ

سورة البقرة الآية : ٣٢



Acknowledgement



First of all, I wish to express my deep thanks to Allah

Almighty who has endowed his help and blessings on me to have the present work achieved in such condition.

I wish to express my gratitude and thanks to Prof. Ashraf A wad Abdel Eltawab, professor of Bacteriology, Immunology, Mycology, for his guidance, criticism, members continuous encouragements and helpful advices including this work.

My grateful thanks to Prof. Amal Mostafa Eid, chief researchs , Prof. Dalia Fathy Khater, chief researchs ,,Animal Healths Researches Institute Tanta branch, Prof. shawky Gisreha professor of pathology , Faculty of Veterinary Medicine, Benha University, for thier encouragements and all guidances during this work.

Finally, I wish to express my warmest gratitude to all members of Bacteriology, Immunology, Mycology Dept., Faculty of Veterinary Medicine (Benha University) and Animal Health Research Institute members.

Thanks, you all for your patience and support.



VITA



- The author was born in September 9, 1981
- In el – Behera governorate, Egypt.
- His primary and preparatory educations was completed in 1993 in Saudi Arabia.

His secondary educations was completed at dalgamoon secondary school, Gharbia Egypt - from which graduated at 1999.

His undergraduate and professional educations was completed in faculty of veterinary medicine, kefir el – sheikh university from which he received a BVSc. degree in 2003 educated qualified members as a general system . Very Good Just the Seventh. He works at animal health research institute, Tanta branch. Under The Supervisions Of Agricultural Researchic Center.



Dedication

My Father

My Mother

My Sister

My Brother

My Uncles

My Family

Always all are supporting religious forever



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Official Scientific File References (DOI)S: A-Universal DOI Records (Zenodo) :**

<https://doi.org/10.5281/zenodo.15584047>.

<https://doi.org/10.5281/zenodo.15583021>

<https://doi.org/10.5281/zenodo.19388186>

<https://doi.org/10.5281/zenodo.17866111>

<https://doi.org/10.5281/zenodo.17745368>

<https://doi.org/10.5281/zenodo.17579555>

National Publication (Egypt – BVMJ)

Published in:

Benha Veterinary Medical Journal (BVMJ)

DOI:

<https://doi.org/10.21608/bvmj.2020.37011.1230>.

EKB Article ID:

116440

Article Link:

https://journals.ekb.eg/article_116440.html.

**[https://docs.google.com/presentation/d/1GhzHKm94jslnJLCxh4mugaamu5Z1E2fX/edit?usp=drive_link
&oid=101122019937214507234&rtpof=true&sd=true](https://docs.google.com/presentation/d/1GhzHKm94jslnJLCxh4mugaamu5Z1E2fX/edit?usp=drive_link&oid=101122019937214507234&rtpof=true&sd=true)**

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LIST OF CONTENTS

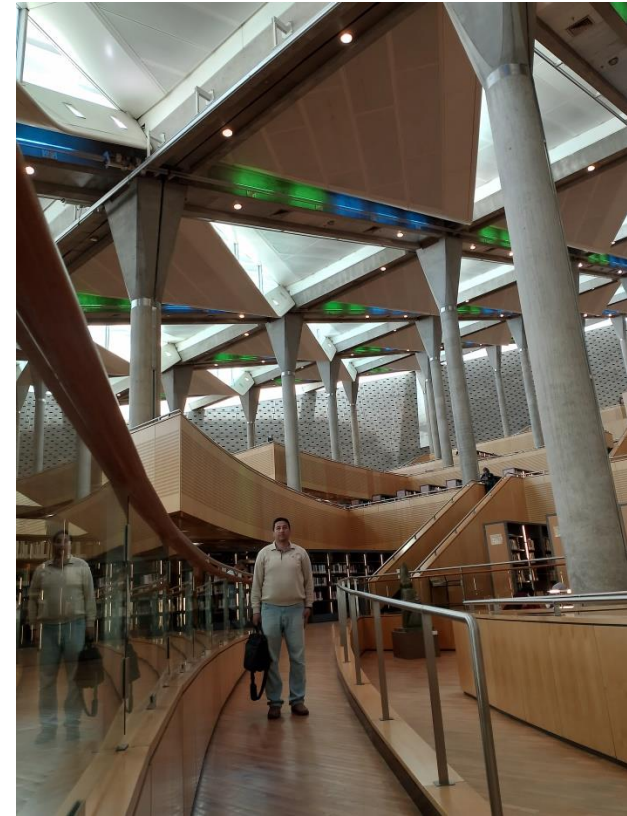


Title	Page
1- Introduction	7-13
2- Reviews of Literatures	14-32
3-Materials and methods	33-42
4- Results.....	38-51
5-Discussion.....	52-61
6- Conclusion and – Recommendations	61-64
7-Summary.....	65-68
8-References.....	69-90
Arabic summary	91-93





INTRODUCTION



1. INTERODUCTION



Food safety is considered one of the most common urgent matters in the food industry worldwide. Spoilage of the food products with foodborne pathogens receive a special concern among food producers, investigators and customers. Consequently, producing a safe food represents one of the most imperative urgencies in the food processing (Friedman *et al.*, [2002](#); Mohamed *et al.*, [2013](#)). The outbreaks of foodborne illnesses caused by *E. coli* have been studied previously in developing countries after ingestion of milk products such as oldstyle cheese which is considered the major source of various types of pathogenic bacteria (Elhadidy and Mohammed, [2013](#)). *E. coli* is one of the major significant bacteria, which has a bad effect on both human and animal species. Thus, this type of bacteria can deteriorate the milk particularly raw milk and other milk products as a result of poor hygienic measures (Lara *et al.*, [2016](#); Garbaj *et al.*, [2016](#)).





Prevention of foodborne illness is a global concern that affects all aspects of food production, including dairy. Dairy cattle are known reservoirs for zoonotic bacteria and milk and milk products have been implicated in outbreaks of foodborne illness (Wells *et al.*, 1991; Zhao *et al.*, 1995; Wallace, 1999). These outbreaks are generally caused by improperly pasteurized products, post pasteurization contamination, or by raw milk and raw milk products.

pathogenic *Escherichia coli* are also present in dairy production environments and have been associated with milk and milk products (Ryser, 2001; Woteki and Kineman, 2003). Although milk can be contaminated with bacterial pathogens via shedding in the udder, these zoonotic pathogens are not commonly associated with mastitis. Fecal contamination is considered to be the most common route of bulk tank milk contamination with these organisms.





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Cheesemaking is a major industry worldwide, and much of it is still practiced on a relatively small scale with only basic hygiene practices (Kousta *et al.*, 2010; Dias *et al.*, 2012).

Among the dairy products, cheese is considered one of the most public sources of vital nutrients (*e.g.* vitamins, minerals and proteins) which represent the main part of healthy food (López-Expósito *et al.*, [2012](#)). In Egypt.



Kariesh is the most popular soft cheese in Egyptian cities and Arabian countries, similar to Domiati (Brown , 2004; Abd-Ehamid , 2012; Hegazy *etal.*, 2012). *al.*,2003).





Dommati cheese is one of soft pickled cheese and makes up about 75% of the cheese produced and consumed due to its nutritional value , convenience and have rich taste ,strong sharp flavor as well as smooth body and texture .





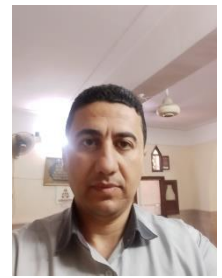
Tallaga cheese is the most popular local type of packaged or unpackaged soft cheeses by all socioeconomic classes in Egypt due to its nutritional values, conveniences and good tastes, clean pleasant creamy low salty taste with a spreadable mellow soft body. It is a product closely related to Domiati cheese and mainly ready for consumption within one month of storage at refrigerator temperature.





Escherichia coli is commonly present in the intestinal tract of humans and animals, and its detection in processed foods is an indication of fecal matter contaminations.





Currently, diarrheagenic *E. coli* strains are classified into seven main pathotypes based on their distinct virulence determinants and pathogenic features, including enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), shiga toxin-producing *E. coli* (STEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusively adherent *E. coli* (DAEC) , Uropathogenic *E. coli* (UPEC) is the most frequent pathogen responsible for up to 80% of UTIs have special virulence factors, including pili or fimbriae, especially type 1 which have the structural gene called fim H gene which mediate attachment to uroepithelial and vaginal cells, resistance to human serum bactericidal activity, haemolysin production. virulence factors of UPEC strains have a significant role in development of UTIs. The most virulence factor dependent upon the UPEC include adhesions type 1 fimbriae encoding by fim H gene (Ruttler *et al.*, 2006; Xia *et al.*, 2010) (Zohreh et al 2014).





Shiga toxin–producing *E. coli* (STEC), also known as verotoxin-producing *E. coli* (VTEC) STEC represent a hazardous public health problem worldwide causing various human gastrointestinal tract diseases, including watery or bloody diarrhea and might develop a life-threatening diseases, such as pretended heamorrhagic colitis (HC), Thrombotic Thrombocytopenic Purpura (TTP) and Haemolytic Uraemic Syndrome (HUS) (Pennington, 2010).





Eae A gene is an intimin secretion gene that enhances attachment and colonization of *Escherichia coli* in the intestinal epithelium, leading to severe cellular destruction and severe infections and severe symptoms like profuse diarrhea and ulcerative colitis.

(*Ibrahim et al.*, 2019).





As soft cheeses (Kariesh, Tallaga and Domiatti) collected from different localities from Gharbia governorate may subjected to contaminations with *E.coli* and may constitute public hazard. *E. coli* constitutes publics hazards of living creatures especially humans (likes meningoencephalitis, pyelonephritis, hepatitis, pneumonitis, pleurisy , pericarditis , peritonitis, osteomyelitis ,appendicitis).

Therefore this work was planned to covers the followings points:

1-Isolations, biochemicals and serologically identifications of pathologically pathotypes of Escherichia coli from kariesh, tallage and domiatti types of cheeses .

2- Molecular identifications of isolated bacteriae (bacterium coli) using PCR techniques.





Review of Literatures





2- Reviews of Literature :

2.3.1 Incidence of *E. coli* of Soft cheese:

Alice *et al.* (2000) isolated *E. coli* colonies from 44 soft white cheese samples and EPEC serogroups O127 and O128 were found in 11.3% of cheese samples.

Pradel *et al.* (2000) reported the isolation of STEC non-O157 strains from cheese in France, all of them carrying the *stx1* gene but not the *eaeA* gene.

El-kosi (2001) examined bacteriologically the kariesh cheese samples and *E. coli* recovered as 100 % of all samples .



Al-Jedah et al. (2001) examined 24 of kariesh cheese produced in Galicia, NW Spain for their microbiological characteristics. They found that *E.coli* was found at low levels of less than 1.72logcfu/g.



Al – Jedah and Robinson (2001) examined microbiologically white brined imported Damietta cheese samples from Egypt and purchased from food stores around Doha (Qatar) and found that all samples were free from *E. coli*.

Ahmed (2002) examined 20 samples of cheese for presence *E.coli* and found that 25 % of the examined samples were contaminated with *E.coli*.

Gomez et al. (2002) examined bacteriologically 114 samples of soft cheese for the presence of Ecoli and founded 0.9 % of samples were positive.



Elmer and Ewina (2003) examined 40 random samples of surface vended kareish cheese collected from different localities in Alexandria and Kafr ElShiekh governorates. *E. coli* could be isolated from 37.5% of examined samples. **Tabet .S (2003)** examined 40 samples of cheese for presence of *E.coli* and found that 20% of the examined samples were contaminated with *E.coli*. the isolated strains were serotyped as O111,O119 and O157.

Spano *et al.* (2003) experimental studies have already shown that treatment at high temperature such as curd stretching at 80 °C for 5 min during the manufacture of cow's milk mozzarella cheese has been shown to effectively control STEC strain growth .





El-Bakery (2004) examined bacteriologically 50 samples of kariesh cheese collected from different markets and shops from giza, Egypt in summer and winter. He found that the percentage of *E.coli* in kariesh cheese was detected in 18 % and 26 in winter and summer respectively.

Moustafa (2004) found that 30.9% of the examined Damietta cheese samples were positive for *E.coli*.

Oksuz et al. (2004) examined 50 white pickled cheeses manufactured from raw milk for the presence of *E.coli* O157 which was detected in 4% of the total cheese samples.

Honiesh et al. (2005) reported that the number of cases deaths 13 in Canada with serotype O175 :H7 of *E.coli* from consumption of contaminated un pasteurized Gouda cheese.





Mussein and Sakuma (2005) studied the role of dairy cattle and their products in human infections with Shiga toxin-producing *Escherichia coli* (STEC). Shiga toxin-producing *E. coli* were isolated from raw milk, milk filters, and dairy products such as yogurt and soft cheeses. STEC infection was attributed to consumption of dairy products that were contaminated with cattle feces. Thus, dairy cattle are considered reservoirs of STEC and can impose a significant health risk to humans.

Nemr, M (2005) isolated *E. coli* from kareish cheese samples by incidence of 24% of the examined samples. The isolated serovars of *E. coli* were O111, O55, O126 and O157:H7 with percentage 25%, 17 %, 33% and 25% respectively.





Sahout and Mostafa (2006) examined 50 samples of kareish cheese collected from local markets in Dakahlia provience, Egypt and found that the examined samples *E. coli* were detected.

Espie *et al.* (2006) reported that there were a number of cases deaths in France due to the contamination of fresh unpasteurized goats cheese by *E.coli* serotype O157: H7 .

Najand and Ghanbarpour (2006) tested 77 samples of domestic soft cheese which were collected randomly from different groceries and markets in Kerman, Iran and they found that 98.70% of cheese samples were positive for *E. coli* and eight EPEC serogroups were isolated from 15 (19.48%) of the samples.

EPEC serogroups included O26 (I), O86 (II), O114 (IV), O142 (IV), O111 (I), O119 (II), O127 (II), and O128 (III).





El-Bessary (2006) examined microbiologically kareish cheese samples and pointed out those *E.coli* were in 60% of the examined samples, respectively.

El-Bessary (2006) examined microbiologically domiatti cheese samples and pointed out *E.coli* were detected in 10% of the examined samples, respectively.

Maha, Al-Ashmawy (2006) examined bacteriologically 25 samples of kareish cheese collected from El-Dakahlia governorate for presence of Coliforms, and reported that coliforms was detected in all examined samples.





et al. (2006) found that *E.coli* causing from gastrointestinal tract-related complications such as diarrhea, dysentery, urinary tract infection, pneumonia and even meningitis.

Najand and Ghanbarpour (2006) tested 77 samples of domestic soft cheese which were collected randomly from different groceries and markets in Kerman, Iran and they found that 98.70% of cheese samples were positive for *E. coli* and eight EPEC serogroups were isolated from 15 (19.48%) of the samples. EPEC sero groups included O26(I), O86(II), O114(IV), O142 (IV and I), O119 (II), O127(II), and O128(III).





Control and Prevention (206) weekly report which reported that during August-September 2005 a total of 52 (2.44%) of 2,160 inmates at a state correctional facility reported diarrhea, including 17 (33%) with bloody diarrhea. Sixteen of the samples were positive for *stx* 1 gene but not for STEC O157 serotyping, and in this case the source of the outbreak likely was an ill food worker.





Dr. Reza (2006) examined Seventy-seven random samples of domestic Iranian soft cheese were collected from different groceries and markets in sterile conditions, cultured in selective media and examined for biochemical tests. Among the 77 samples, *E coli* was isolated from 76 (98.70%) of them and 15 (19.48%) of the isolates were EPEC. Eight EPEC identified serogroups were included: O26(I), O86(II), O114(IV), O142 (IV and I), O119(II), O128(III), and O127(II). All these types are pathogens. O127 was the most prevalent serogroup, followed by O128 and O119. The occurrence of a high proportion of *E coli* in our cheese samples may be due to lack of proper sanitation and absence of pasteurization of milk used for cheese making. Therefore, stringent hygienic measures must be followed and pasteurization of milk should be imposed to prevent contamination of cheese with coliforms, thus avoiding additional outbreaks of food-borne illness caused by *E coli*.





Rey *et al.* (2006). showed that 3 STEC carried *stx1* gene, 5 possessed *stx2* gene, and 1 both *stx1* and *stx2*. Whereas only O157:H7 serotype showed *eae* virulence gene.





Baraheem *et al.* (2007) examined 80 samples of kareish cheese collected from Alexandria government. The prevalence of fecal coliforms and *E.coli* reached 80% and 75%, respectively.

Lothar Beutin *et al.* (2007) we examined 219 shiga toxin producing *Escherichia coli* (STEC) strains from meat , milk and cheeses samples collected in germany between 2005 and 2006 and they found that shiga toxin Stx1 present in 88 (40 %) strains .





...milk cheese from
different supermarkets in Middle Western Brazil and were analyzed for *E. coli* which were recovered from 48 (96.0%) of the samples. The identified serogroups were O125 (6.0%), O111 (4.0%), O55 (2.0%) and O119 (2.0%). Three (6.0%) and 1(2.0%) of *E. coli* isolates were verotoxogenic *E. coli* (VTEC) and enterotoxogenic *E.coli* (ETEC), respectively.

***Omer et al.* (2007)** analyzed 80 samples of kariesh cheese collected from supermarkets shops and street vendors in alexandria, Egypt for bacteriological quality. The examined samples showed that E coli was





Almeida *et al.* (2007) stated that out of 70 cheeses samples were examined for presence of *E.coli*, O157, for grading them as satisfactory, acceptable, unsatisfactory, or unacceptable and potentially hazardous; 22 were graded as satisfactory or acceptable, 37 were considered unsatisfactory because of the presence of *E.coli*.

El-baradei *et al.* (2007) examined microbiologically Damietta cheese samples and they found that 90% of the samples were positive for *E.coli*.





Garofalo *et al.* (2007) studied 18 UPEC isolates collected from females and found that the *fimH* gene was the most prevalent virulence factor and 100% of the isolates had that gene.

Irino *et al.* (2007) It is also important to mention that in Brazil, human STEC infections have been primarily associated with diarrhea and most of the STEC strains isolated are non-O157 strains harboring *stx1* and *eae* genes .





Nagachinta and Chen (2008) reported an association between virulence factors and antimicrobial resistance in *E. coli* strains isolated from dairy cows.

Osman and Omer (2008) found that the exact examined white soft cheese were positive for the prescence of *E. coli*.





Hassan (2008) reported that microbiological quality, composition and some chemical properties were determined in 40 samples of kariesh cheese randomly collected from various markets located in Cairo. Microbiological examination revealed that 12% of the examined Kariesh cheese were contaminated with *E. coli*.

Sabry and Laila et al. (2008) examined bacteriologically a total of fifty raw milk and twenty-three Kareish cheese samples were randomly collected from different shops and distributors in Qena city. *E. coli*

16 out of 41 (47.98%) of Kareish cheese samples were





Fadel and Jehan (2009) collected 40 Kareish cheese samples from different localities in Ismailia city and they could isolate 4 different *E. coli* serovars STEC group (O128:H₂), EPEC (O127), EIEC group (O124:H7) and one untypable strain.





Ali *et al.* (2009) examined 15 random samples of kariesh cheese from dairy shops and street vendors in rural areas for Microbiological evaluation. The incidence of *E coli* was 6.6% out of examined samples.

Mladin *et al.* (2009) evaluated the distribution of virulence genes among the studied UPEC and showed that the prevalence of different virulence genes varied from 10% for the *cnf* gene to 80% for the *fimH* gene.





Moras (2009) fifty-five samples of kariesh cheese were collected from different non inspected commercial establishments and submitted to inspection of *E coli* it were detected.

El-prince, Enas *et al.* (2010) examined 30 random samples of kariesh cheese were collected from assuit city, Egypt for fecal pollution indicators. All examined samples were contaminated with coliforms. The incidences of *E coli* in kariesh cheese were 33.3%.





Kousta *et al.* (2010) Cheese can be easily contaminated by food handlers who practice poor personal hygiene at some point during cheese preparation and/or assembly line.

Ortolani *et al.* (2010) studied the microbiological quality and safety of 18 soft cheese samples and they found that low levels of *E. coli* was observed.



Todd *et al.* (2010), several foodborne outbreak of colibacillosis investigation reports have identified the hands of food workers as the source of pathogens in the implicated food.

Watts *et al.* (2010) demonstrated that the *fimH* gene was the most frequent virulence gene and was detected in 98% of *E. coli* strains isolated from patient with UTIs.





Cergole-Novella *et al.* (2011) showed that the highest resistance rates of STEC were identified for tetracycline (100%), streptomycin (78%), and trimethoprim-sulfamethoxazole (56%). Furthermore, all strains were harbored *stx* genes.

El sayed *et al.* (2011) examined microbiologically (30 samples of domiatti cheese and 15 samples of tallaga cheese and 10 samples of kariesh cheese) were collected from Cairo and Giza governorates for the presence of *E.coli* and results respectively were (20%, 33,3%, 26.6%).



microbiologically 120 traditional fresh cheese samples for the presences of *Escherichia coli* in Iran state and the result were four isolate from traditional cheese and the genes Stx1 and Stx2 were detected three *E.coli* isolates obtained from traditional cheeses samples.



Zeinhom (2011) tested 50 random samples of kariesh cheese were collected from different localities in beni-suef Governorate, Egypt monitoring of enteric pathogen with *E coli* the data showed that *E coli* was not isolated from any of the examined samples of kariesh cheese.



Hosny *et al.* (2011) made a survey to evaluate the level of microbial contamination of 35 samples of Kareish cheese retailed in Cairo area and the results indicated that *E.coli* as fecal indicator, was isolated from 25.7% of the total Kareish samples.

Ozen *et al.* (2011) studied the microbiological quality of white pickled cheese produced in small dairy plants which was investigated and found *E.coli* was isolated from 40% of the samples .



microbiologically 115 samples of white cheeses from 3 different areas in Baghdad surveyed and examined for the presences of *Escherichia coli* the result showed 7 samples are positive for the presence of *Escherichia coli* and one strain contain Stx1 virulence gene.

Dias *et al.* (2012) reported on the implementation of Good Manufacturing Practices (**GMP**) in a small processing unit of mozzarella cheese in Paraná, Brazil, and that the primary hurdles for the implementation of **GMP** were related to the difficulties to change hygiene practices of food handlers.



microbiologically 25 samples of domiatti cheese and 25 samples of tallaga cheese and 25 samples of kariesh cheese collected from dairy shops and street vendors in Beni Suif city for the presence of *E.coli* and the result were 30. 48%, 27.80% and 39.94% respectively.

Abd El-latif, eman (2012)

examined bacteriological 25 samples kariesh cheese randomly collected different farmers in El-behira, Egypt and examined microbiologically. The uthor found that *E.coli* was detected in 44% in kariesh cheese.



Ahmed (2012) examined 25 random samples of kariesh cheese were collected from different markets and retail shops in Beni-Suef city, Egypt for chemical and microbiological examination. The prevalence rate of E coli isolation was 28.34%.

Arabi *et al.* (2012) investigated the frequency of FimH and other adhesions genes in UPEC and determined the fimH gene frequency as 87.7%.



markets, and on-line sources and the samples were then analyzed for the presence of Salmonella, *E.coli* and *S. aureus*. The results revealed that 2 of those contained *E.coli* and 3 other samples contained *S.aureus* but Salmonella was not detected.

Nagah *et al.* (2012) examined 120 random samples of raw milk, Damietta cheese, kareish cheese and cooking butter (30samples each) which were collected from different localities in Assiut city, Egypt and they found that the incidence rates of *E. coli* in raw milk, Damietta cheese and kareish cheese samples were 83 %, 30% and 60 %, respectively.



cheese samples that were collected from different localities in Alexandria and the results revealed that the rate of isolation of *E. coli* was 26%.

Mohammed El-hadiddy and Mahmoud ahmed(2012) Examined microbiologically 124 raw milk cheeses samples (64 kariesh and 60 damietta cheeses samples) for the prescences of STEC using molecular detection of virulence markers such as shiga toxin (Stx1) and intimin gene (eae) and by serotyping they found onley 14 samples positive for the *E.coli*.



Elhadidy and Mohammed (2013) studied microbiologically 124 raw milk cheese samples (kareish and Damietta cheese samples) for *E.coli* contamination. They found that 14 *E.coli* strains were positive for STEC.

who reported that STEC incidence in 124 cheese samples was 11.29%. All recovered isolates harbored *stx2* gene 100% either single or in association with the *stx1* gene, while *stx1* was present in 64.28% of isolates. They reported that intimin gene (*eae*) was present in 21% of isolates.



Claeys *et al.* (2013) reported the occurrence of a number of pathogenic bacteria in raw milk and their products, including pathogenic *E. coli*. Therefore, consumption of raw milk and traditional milk products can be caused foodborne pathogens outbreaks.

Tarchouna *et al.* (2013) reported that among the studied virulence genes of UPEC strains, the fimH gene was the most prevalent virulence gene and was found in 68% (61/90) of the UTI isolates.



Claeys *et al.* (2013) reported the occurrence of a number of pathogenic bacteria in raw milk and their products, including pathogenic *E. coli*. Therefore, consumption of raw milk and traditional milk products can be caused foodborne pathogens outbreaks.

Tarchouna *et al.* (2013) reported that among the studied virulence genes of UPEC strains, the fimH gene was the most prevalent virulence gene and was found in 68% (61/90) of the UTI isolates.



28% of samples were positive for *E. coli* were collected from milk vendors, retail shops located in Anand city, under aseptic precautions. For the enrichment of the organism from the collected samples, MacConkey broth was used and inoculation was carried out on MacConkey agar and EMB agar. Later on, to confirm the isolates, various biochemical tests such as IMViC test, Urease test were performed. Evaluation of antibiotic sensitivity pattern of *E. coli* was assessed by disk diffusion method. Finally the *E. coli* isolates were screened for the presence of virulence associated genes by PCR and founded that 7 isolates of *E. coli* positive for the presence of Stx1 (8.75 %) only and 5 isolates were positive for the the presences of both Stx1 and Stx2. 7 isolates were positive for *eae A*.



samples of unpasteurized soft cheeses from retail shops which were analyzed for detection of *E.coli* and found 24 of cheese samples were positive for *E.coli*.

Gundogan and Avci (2014) examined samples of white soft cheese from three different dairy-processing plants in Ankara to find out if they were contaminated with *E.coli*. The results showed that the contamination percentages of *E.coli* were 60%.

Abo Zeed (2014) examined microbiologically 30 samples of kariesh cheese randomly collected from different dairy shops and supermarkets in cairo, Giza and Kalyobia governorate, Egypt. The results revealed that *E.coli* failed to be detected.



Dkura & Marin (2014) examined 330 *E. coli* strains isolated from 111 Minas frescal cheeses from Southwest Minas Gerais State and also did not detect *stx* 1, *stx* 2, or *eae* genes among the *E. coli* isolates.

El- kholy *et al.* (2014) examined bacteriologically 50 fifty samples of domiatti cheese were randomly collected from supermarkets ,retailers and shops in Beni suif governorate , Egypt for the presence of *Escherichia coli* and the result were 13 samples positive by percentage 26%.



Mussain *et al.* (2014) collected 12 samples of different dairy products including cheese from different localities of Lahore and found 54% of tested samples were contaminated with *E.coli*.

Walid *et al.* (2014) examined bacteriologically 40 samples of domiatti cheese and 20 samples of kariesh cheese samples for the presence of *Escherichia coli*



Al Jobori *et al.* (2015) examined 70 samples of white were as 87%.

Assel A. Saeed (2015) examined a total 50 cheese samples was collected from market in al-diوانيya city the study was done in period between august and October 2014 and recorded 38 samples were positive for *E.coli*.



Assumpção *et al.* (2015) reported from 561 *E. coli* isolates, 90 (16.0%) were carriers of *stx1*, 97 (17.3%) of *stx2*, and 45 (8.0%) of *eae* genes singly. 37 (6.6%) isolates were carriers of *stx1* and *stx2*, 110 (19.6%) were carriers of *stx1* and *eae*, and 67 (11.9%) were carriers of *stx2* and *eae*.



Metwally and fatma (2015) examined about 25 samples of kariesh cheese collected from retailers and small dairy shops in giza and beni-suef governorates markets for the presence of *E. coli* and result founded that about 72% of samples were positive for the *E.coli*.

Mohamed (2015) examined kariesh cheese bacteriologically for the presence of *E.coli* and it is recorded from the all samples by



Elnahas *et al.* (2015) examined microbiologically 40 samples of kariesh cheese samples were collected from different localities in El Monofia governorate for the presence of E.coli and founded 50 % of samples were positive for pathogenic E.coli.

Gamal *et al.* (2015) examined microbiologically 50 samples of domiatti cheese and 30 samples of kariesh cheese were collected



El bajory *et al.* (2015) examined microbiologically

40 samples of kariesh cheese and 40 samples of domiatti cheese collected from supermarkets and dairy shops and street vendors in El Gharbia governorate for the prescence *E.coli* and the result were 52.5 % and 15% respectively.

Jehan *et al.* (2015) examined microbiologically 30 samples of kariesh cheese and 30 samples of domiatte cheese from different shops and super markets and street vendors in Ismalia city for the prescence of E coli and the results were 9 samples of kariesh cheese were positive and 12 samples of domiatti cheese were positive.



Zohreh Hojati *et al.* (2015) A total of 140 isolated *E. coli* strains from patients with UTI were identified using biochemical tests and then evaluated for the FimH gene by polymerase chain reaction (PCR) analysis. The FimH gene was found in 130 isolates (92.8%) of the UPEC strains. Of 130 isolates positive for the FimH gene, 62 (47.7%) and 68 (52.3%) belonged to hospitalized patients and outpatients, respectively. The results of this study indicated that more than 90% of *E. coli* isolates harbored the FimH gene. The high binding ability of FimH could result in the increased pathogenicity of *E. coli*; thus, FimH could be used as a possible diagnostic marker and/or



Abo baker *et al.* (2016) isolated from fresh locally made soft cheese were 9 (33 %) and 3 (11 %) respectively from (maasora and Ricotta soft cheese) positive for the prescence of E.coli .

Ombarak *et al.* (2016) examined 55 kariesh cheese samples for the prescence of Ecoli and found that 41 samples (74.4%)were positive for E.coli occurrence and this isolates screened by PCR for the prescence of virulence genes Stx1 and eae virulences genes and the percentages were (,90 %),(,45%) respectively .



Amal and soad (2016) examined 50 samples of domiatti cheese and 50 samples of kariesh cheese in cairo and giza governorates for the presence of the E.coli and they found that the results were negative.

Nabil *et al.* (2016) examined 95 food and environmental samples which were examined for the presence of shiga toxin-producing *Escherichia coli* for the presence of Stx1 and



Sameh a wad (2016) examined microbiologically 15 samples of traditional kariesh cheese made using raw milk were collected from different markets in different governorates (Alexandria, Behera, kefir el sheikh, domiatte, Gharbia) for the presence of *E.coli* and the results were negative.



Lofty *et al.* (2017) examined 25 samples of each kariesh cheese and domiatte cheese collected from Mansoura city for the presence of *E. coli* and found the results were 32% and 16% by using MacConkey agar medium and 44% and 20% by using EMB medium. Furthermore, the results showed that 11 out of 11 (100%) *E. coli* had both *stx1* and *stx2* while *eae* gene was not found in *E. coli* isolated from traditional milk products. The *eae* gene is an accessory virulence factor for STEC that is thought to enhance the virulence of STEC, while some STEC strains not harboring *eae* gene have been shown to cause human illnesses.



Chaleshtori *et al.* (2017) examined microbiologically 26 cheeses samples randomly purchased from local markets in Kashan, Iran and evaluated for the presence and occurrence of STEC by culturing and molecular methods and the incidence of *E. coli* in 26 samples was 11.54 %. Further more isolates were assayed for the presence of shiga toxins Stx1 and intimin gene (*eae*). The findings showed that 100 % of *E. coli* contain Stx1 while *eae* gene was not found in *E. coli* isolates of traditional milk products. In recent years, Shiga toxinogenic *E. coli* (STEC) strains as emerging groups of foodborne pathogens are responsible for most foodborne illnesses. The pathogenicity of these strains is almost related to the ability of the microorganism to produce the one or two cytotoxins called Shiga toxins (*stx1* and *stx2*), and the presence of the intimin (*eae*) gene, which is responsible for intimate attachment of the organism to the intestinal epithelium.



Douellou *et al.* (2017) demonstrated that the virulence gene profiles of dairy products and human STEC strains were similar to **Farhad shafati chaleshtori *et al.* (2017)**.

Patricia cardosa and jose moacir marine (2017) examined 59 raw milk cheese samples collected from local producer in brazil for microbiological analysis and found that 38 (64,4%) tested positive for E.coli . then 147 strains of e.coli from positive samples and screened by polymerase chain reaction for the prescence of virulence genes Stx1 (verotoxin type 1) and eae (intimin) gene and found that (9,5 %) were positive for the prescence the Stx1 gene and 11 of them also harboured the eae gene . all STEC – contaminated food can be considered potential hazardous .



Hussien et al. (2019), inspected 75 samples of kariesh cheese collected from different presented in monofia governorate According to the results, the frequency of *E. coli* isolated Kariesh was 16%. Serological identification classified the *E. coli* strains into two groups enterohemorrhagic *E. coli* (EHEC) serogroup(O26: H11, O91: H21, O111: H2 and O103: H2). V the enterotoxigenic *E. coli*(ETEC) serogroup were detected as O125: H21 which is the most prevalent strain. O171: H2, O86 and O119: H6 belonging to enteropathogenic *E. coli* (EPEC). The prevalent gene detected in *E. coli* strains was *stx1* (87.5), *fimH* (75) and *eaeA* (25%) genes. In conclusion, Kariesh cheese are extremely tainted with pathogenic *E. coli* strains, which represent a strong hazard on the human health.

Taha et al. (2019) examined microbiologically 90 cheese samples high salt cheese, kariesh cheese and Tallga cheese were examined for E.coli. Serodiagnosis of E.coli has been done by slide agglutination test. Toxogenic E.coli isolates were detected using PCR assay. Results postulated that the detection rate of non O 157 was 36,33% in Kariesh cheese, 20 % in high salt cheese and 33,33% in Tallaga cheese. Ten different serotypes of E.coli non O157 Serotypes of E.coli non O 157 have been identified as follows: O1,O18,O20,O25,O26,O125,O126,O127 and O157. Regarding PCR results: all serogroups were negative for *Stx1* gene, only serogroup

Ibrahim *et al.* (2019) examined bacteriologically 30 samples of Domiatti cheese and 30 samples of kariesh cheese randomly collected from local markets , farmers vendors and supermarkets at different localities in Nile Delta region , Minoufia governorate ,Egypt . E.coli recovered from samples were 13 (43,3 %) and 22 (73.3 %) respectively . The Serotyping reveals in Kariesh cheeses as following O128;H2,O26;H11, O44 : H 18 , O119 :H6, O127:H6, O127: H6, O111:H2, O124 , O146:H21 and in Domiatti cheese as following O128: H2, O26 : H11, O91 :H21, O127:H6 , O111:H2, O124, O146 H41and PCR rseults as following O26;H11 had *stx1* and *eae A* genes , O44 : H 18 hadnot *stx1* and *eae A* gens , O91 :H21 had *stx1* and had not *eae A*, O111:H2 had *stx1* and *eaeA* genes , O124 had not *stx1* and *eaeA* genes .

Ibrahim *et al.* *et al.* (2020) examined non O 157:H 7 shiga toxin producing Esherichia coli (STEC) is amajor food borne pathogen that causes sever disease in humans ranged from mild gastroenteritis to critical illness and death especially O 26 . O 111 in kariesh cheeses .







Materials and Methods

(Virpari *et al* 2013, lotfy *et al* 2017 and Ibrahim *et al. et al.* 2020)



3-Materials and Methods: (Virpari *et al* 2013, lofty *et al* 2017 and Ibrahim *et al. et al.* 2020)

The study was undertaken to isolate pathogenic *E.coli* from various types of cheese .

3.1. Sampling:

One hundred and fifty random samples of soft cheese (50 samples of kariesh cheese – 50 samples of tallaga cheese – 50 samples of domiatti cheese) were collected randomly from small dairies, local super markets, farmers house, shops and street vendors in different localities at Gharbia Governorate. The collected samples were transferred directly to the laboratory in an ice – box under aseptic conditions without delay and then subjected to bacteriological examination.

The bacteriological examinations of samples were carried out in Animal Health Research Institute Tanta, while Serological and Molecular examinations was done in Animal Health Research Institute Dokki.



3-2. Bacteriological examinations:



5 grams of samples of each (kariesh samples – tallaga cheese- domiatti cheese) were added to 25ml sod. Citrate and blended for two minute for examination of *E.coli* by this tests. (Preparation of samples: (maintain PH)

3-2-1.1.maconkeys broth test (acid and gas test): wo ml of each blended samples are added to MacConkey broth with ibin Dorf tubes and incubated for 24hours at 37°C for the detection of acid and gas if it is present indicated for the presence of *E.coli*.

3-2-1.2. Eosin methylene blue media test:

From the positive MacConkey's broth test with yellow colorations and acid and gas results are cultured on the Eosinmethylene blue media Eosin Methylene Blue (EMB) agar plates, which it is selective media for *E.coli* ,then incubated at 37 °C for 24 hours. Typical colonies of *E. coli* appeared greenish metallic with or without dark purple center. Suspected colonies were picked up and transferred to nutrient agar slopes and then incubated at 37 °C for 24 hours. The purified colonies were subjected for

3-2-2. Biochemicals Identifications:

The pure colonies of isolates were identified biochemically as fellow :-



3.2.2.1- Indol test:

To each 48hrs incubated culture at 37°C in 1% peptone water, one ml of ether was added. The tubes were vigorously mixed and allowed to stand for few minutes until the ether rise to the surface. About 0.5ml of Kovac's reagent was added on the side of the tube gently. Development of rosy color ring indicated presence of indol.

3.2.2.2- Methyl red test:

To each 5ml of 48 hours glucose phosphate broth culture incubated at 37°C, 5 drops of methyl red reagent were added. Red color indicates a high hydrogen ion concentration (positive reaction); while a negative one gives a yellow color.



3.2.2.3- Voges-Proskauer (V.P.):

To 5 ml of 48hrs glucose phosphate broth culture incubated at 37°C for 3 days, 3ml of alcoholic solution of alpha-naphthol, and 1 ml 40% potassium hydroxide solution were added. The mixture was thoroughly shaken and examined after 15 minutes and one hour. A positive reaction is indicated by the development of a strong red color.

3.2.2.4- Citrate utilization test:

The ability to utilize citrate was detected by making a single streak over the surface of Simmon's citrate agar slope, then incubated at 37°C for 7days. The development of blue coloration indicated citrate utilization.



3.2.2.5- Urease test:

This test was carried out by means of Christensen's medium in order to test the ability of isolated organisms to hydrolyze urea. The isolated strains were inoculated onto the surface of the slope of urea medium and incubated at 37°C to be examined after 4hrs and daily for 5 days. Development of pink color due to splitting of ammonia which acted on phenol red indicator indicated hydrolyzes of urea.

3.2.2.6- H₂S production:

Triple sugar iron (TSI) agar was used for this test. A dual type of inoculation was made from a purified solid culture medium, firstly by stabbing the butt and secondly by streaking the slope. Incubation takes place at 37°C and examined daily up to 7 days for blackening due to H₂S production. The color of the button and slant, also the gas formed should be observed and judged.



3.2.2.7- Eijkmann's test: (Growth at 44)

MacConkey's broth tubes provided with Durham's tubes were used. The tubes were kept in a water bath adjusted at $44 \pm 5^{\circ}\text{C}$ for at least 24 hours before inoculation with the suspected organism. Inoculated tubes were returned immediately to water bath and incubated for 48hrs at 44°C . Production of acid and gas indicates positive result.



3.2.3-Serological Identification of *E.coli* serovars according to (Kok *et al.*, 1996).

- Two separate drops of saline were put on a glass slide and a portion of the colony from the suspected culture was emulsified with the saline solution to give a smooth fairly dense suspension.
- To one suspension, control, one loopful of saline was added and mixed. To the other suspension one loopful of undiluted antiserum was added and titled back and forward for one minute.
- Agglutination was observed using indirect lighting over a dark background. When a colony gave a strongly positive agglutination with one of the pools of polyvalent serum, a further portion of it was inoculated onto a nutrient agar slant and incubated at 37C for 24 hours to grow as a culture for testing with mono-valent sera.
- A heavy suspension of bacteria from each slope culture was prepared in saline, and slide agglutination tests were performed with the diagnostic sera to identify the O-antigen.



3.2.4-In-Vitro antibiotic sensitivity of E.bacteriae:

Subcultures from the isolates were prepared and the test was applied as follows:

A smooth single colony was inoculated in 5 ml nutrient broth and incubated at 37°C for 18 hrs, then turbidity was adjusted to 0.5 McFarland contain (1.5×10^8) colony forming unit/ml, then few drops of the inoculated broth were flooded on to the surface of Muller-Hinton agar plates. Excess of cultural fluid was removed aseptically and the plates were allowed to stand for 15 minutes at 37°C for dryness. Then the inoculated plates were over laid with antibiotic discs using a sterile forceps, the discs were distributed in a manner where the distance among them was optimum and away from the edge of plate to avoid overlapping of inhibition zones and give more wide area for the zone of inhibition. The inoculated plates were incubated at 37°C for 24 hours. The Inhibition zones, in mm were measured and scored as sensitive, intermediate and resistant categories with the critical break points recommends by By My Hand CLSI (CLSI, 2018).



3.2.5-Polymerase Chains Reactions Techniques (PCRTs):

3.2.5.1- Materials used for extractions of DNAe:

3.2.5.1.1-QIAamp DNA Mini Kit Catalogue no.51304 .

The QIAamp DNA Mini Kit provides silica-membrane-based nucleic acid purification from different types of samples. The spin-column procedure did not requires mechanical homogenization, so total hands-on preparations time is only 20 minutes.

3.2.5.1.1-Ethanol 96% Apply chem.

3.2.5.2-Equipments and apparatuses used for extractions of nucleic acids:

3.2.5.2.1-Epindorff tubes 1.5 ml capacity.

3.2.5.2.2--Monochannel micropipettes 20-200 μ l, 100-1000 μ l (Biohit**).**

3.2.5.2.3--Sterile filter tips. (200 μ l ,1000 μ l) capacity.

3.2.5.2.4-Centrifuge (Sigma sartorius**).**

3.2.5.2.5-Type II-A bio safety cabinet (Thermo**).**



2.5.3-PCR Master Mix used for cPCRTs.

3.2.5.3.1-Emerald Amp GT PCR master mix (Takara)Code No.

RR310A

Contains:

A) Emerald Amps separates GT PCR master mix (2x premix).

B) PCR grade water

C)Oligonucleotides primers used in cPCRTs.

They have specific sequence and amplify a specific product

as shown in Table **(primers-securities imported**

biokeys).

Table (specific not copied): Oligonucleotide primers sequences

Source: Metabolon (Germany). **(supposes nation**

manufactures).

Genes	Sequences	Amplified products	References
Stx1	ACACTGGATGATCTCAGTGG	614 bp	Dipineto et al., 2006
	CTGAATCCCCCTCCATTATG		
fimH	TGCAGAACGGATAAGCCGTGG	508 bp	Ghanbarpour and Salehi, 2010
	GCAGTCACCTGCCCTCCGGTA		
eaeA	ATGCTTAGTGCTGGTTTAGG	248 bp	Bisi-Johnson et al., 2011
	GCCTTCATCATTTGCTTTC		



3.2.5.4-DNA Molecular weights markers :-

3.2.5.4.1-Gel Pilot 100 bp plus ladder (cat. no. 239045) :-
supplied from QIAGEN (USA).

A) - Number of bands: 11

B) - Sizes ranges : 100-1500 bp.

C) - Genes rulers : 100 bp DNA ladder (cat. no. SM0243)supplied from ferments



3.2.5.5-Materials used for agarose gels electrophoresis:

(falsas negatives-available)

3.2.5.5.1- Agarose 1.5% (Sambrook *et al.*, 1989)

A multi-purpose, high gels strengths agarose suitable for a wide ranges of molecular biologics techniques (Not The Reals Times Techniques) . As It has high gel strength and limited differentiated limits, multi Agarose could effectively separate large DNA fragments with reduced running times. This in turn means less band diffusion, a problem often associated with long running times. (costed - delaying- not honesty- not useful in emergencies and wide ranges of victims)

It was prepared as follow:

A) - Agarose powders (AP genes) : 1.5 g.

B) - TBE : 100 ml.

D) - Ethidium bromide solution 10 mg / ml (Sambrook *et al.*, 1989)

i) - Ethidium bromide powders (Sigma) 10 mg.

ii) - Sterile DW 1.0 ml.

It was mixed and stored covered at 4°C

It was added to melted agarose to reach a final concentration of 0.1-0.5 µg/ml.



C) - Tris borate EDTA (TBE) electrophoresis buffer (1x) (WHO, 2002) :

i) - Tris buffer (Fluka) : 10.78 g.

ii) - Boric acid (Fluka) : 5.5 g.

iii) - EDTA for DNA Fragments easily separations (Winlab) : 0.82 g .

It was brought up to 1 liter with de ionized water, pH was checked up. If the pH was out of the range of (8-8.6) , a new solutions were surely preparing .

Any changes in ions concentrations will affects the migrations of the DNA Fragments through the gel.



3.2.5.6-Equipment and apparatuses used in cPCR

- i)- Calibrated cylinders.
- ii)- Glass flasks.
- iii)- CR tubes 0.2 ml capacity.
- iiii)- (Scales tec).
- iv)- Microwave (Panasonic).
- v)- mono channels micropipettes (2-20 μ l) (Biohit).
- vi)- Sterile filter tips.
- vii)- Gel casting apparatus (Biometra).
- viii)- T3 Thermal cycler (Biometra).
- viii)- Power supply (Biometra).
- viii)- Type II A biosafety cabinet. (Thermo).
- x)- Gel documentation system. (Alpha Inno tech).
- xi)- Deionizer (Millipore).
- xii)- Double distillatory (Sanyo). (Romanians assays).



3.2.5.7-Methods :-

1- Extractions of DNAe accordings to QIA amp DNA mini kits instructions:

1. 20 μ l QIAGEN protease were pipetted into the bottom of a 1.5 ml microcentrifuge tube.
2. 200 μ l of the sample was added.
3. 200 μ l buffer AL were added to the sample, mixed by pulse vertexing for 15 seconds.
4. The mixture was Incubated at 56°C for 10 min.
5. The 1.5 ml microcentrifuge tube were centrifugated to remove drops from the inside of the lid.
6. 200 μ l ethanol (96%) were added to the sample, and mixed again by pulse vertexing for 15 seconds. After mixing, the 1.5 ml microcentrifuge tube was briefly centrifugated to remove drops from the inside of the lid.
7. The mixture from step 6 was carefully applied to the QIA amp mini spin column (in a 2ml collecting tube) without wetting the rim. the cap was closed, and centrifugated at 8000 rpm for 1 min. The QIA amp mini spin column was placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded.



1. The QIA amp mini spin column was carefully opened and 500 μ l buffer AW1 were added without wetting the rim. The cap was closed, and centrifugated at 8000 rpm for 1 min. The QIA amp mini spin column was placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded.
2. The QIA amp mini spin column was carefully opened and 500 μ l buffer AW2 were added without wetting the rim. The cap was closed, and centrifugated at full speed for 3 min.
3. The QIA amp mini spin column was placed in a new 2 ml collection tube and the old collection tube was discarded with the filtrate. Centrifugation at full speed for 1 min was done.
4. The QIA amp mini spin column was placed in a clean 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate was discarded. The QIA amp mini spin column was carefully opened and 100 μ l buffer AE were added. The QIA amp mini spin column was Incubated at room temperature (15-25°C) for 1 min, and then centrifugated at 8000 rpm for 1 min.



2- Preparations of PCR (s) Master Mix according to Emerald Amp GT PCR master mix (Takara) : Code No. RR 310A kit .

3- Cycling conditions of the primers during cPCR.

Temperature and time conditions of the two primers during PCR are shown in Table (paid specifics) according to specific authors and Emerald Amp GT PCR master mix (Takara) kit .

Table (paid specifics): Cycling conditions of the different primers during cPCRs Techniques :-

Component	Volume/reaction
Emerald Amp GT PCR master mix (2x premix)	12.5µl
PCR grade water	4.5 µl
Forward primer(20 pmol)	1 µl each
Reverse primer (20 pmol)	1 µl each
Template DNA	6 µl
Total	25 µl

genes	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	e
Stx1	94°C 5 min.	94°C 30 sec.	58°C 40 sec.	72°C 45 sec.	35	
fimH	94°C 5 min.	94°C 30 sec.	50°C 40 sec.	72°C 45 sec.	35	
eaeA	94°C 5 min	94°C 30 sec	51°C 30	72°C 30	35	7



ghts markers: -

ntly by pipetting up and down. 6 μ l of the required ladder were directly

oreses (Sambrook *et al.*, 1989) :-

tion)) – (Idiots Every Where) :-

rose (1.5 g) was prepared in 100 ml TBE buffer in a sterile flask, it wa

, and allowed to cool at 70°C, then 0.5 μ g/ml ethidium bromide was ad

oured directly in gel casting apparatus with desired comb in apposition

ved, and the electrophoresis tank was filled with TBE buffer. Twenty

ex PCR product, negative control and positive control were loaded to t

h. The run was stopped after about 30 min and the gel were transferred

ed by a gel documentations systems and the data were analyzing's thro





RESULTS



RESULTS

The morphological characters of the colonies on Eosine Methylene Blue were brilliant green. A total of 44 bacterial isolates were obtained from examined samples, 27 bacterial isolates from kariesh cheese, 21 bacterial isolates from domiatti cheese, 16 bacterial isolates from tallaga cheese. *E. coli* isolated from kariesh cheese with an incidence (54%) as shown in (Table 3).

E. coli isolated from domiatti cheese with an incidence (42%) . *E. coli* isolated from tallaga cheese with an incidence (32%) as shown in (Table 3).

E. coli give positive results to Indole test : positive (My Hand),, Methyl red : Negative, Vogues-Proskauer : Negative and simmons citrate : Negative

My Hand), Eijkman's test : positive . *E. coli* gives yellow slant and yellow bottom without H₂S production .

E. coli isolates were serotyped, four from kariesh cheese and one from tallaga cheese, serological identification revealed that five isolates from kariesh cheese were belonging to (O119 :H7, O111 :H8, O15 :H7, O18: H1 :K7 and O128). One isolates from domiatti cheese belonging to (O124) .



Table (1): Antimicrobial discs, concentration or antibiogram profile of antibiotics susceptibility of their actions on the isolated *E.coli* :

Susceptible (mm)	Intermediate (mm)	Resistant (mm)	Sensitivity disc content (µg)	Antimicrobial agent
18 or more	13-17	12 or less	30	Chloramphenicol (C)
20 or more	15-19	15 or less	5	Ciprofloxacin (CP)
23 or more	14-22	13 or less	15	Erythromycin (E)
4 or more	1-2	0,5 or less	5	Enerofloxacine(ENR)
17 or more	13-16	12 or less	30	Neomycin (N)
128 more	64	32 or less	300	Neurofurantoine (F)
19 or more	15-18	14 or less	30	Oxytetracycline(OT)



Table(2) :PCR Table:- characterization of *E.coli* by PCR from collected soft cheese samples:

Number of E.coli isolates	Percentage of Stx1 gene presence (Shiga toxin)	Percentage of eae A gene presence (Intimin gene)	Percentage of Fim H gene presence (The structural rule of type 1 fimbriae in UPEC)Formation of Attachment and biofilm infection of E.coli
7	All negative – (Zero %)	Two isolates positive number (3,4) other isolates were negative(28.5 %)	All were positive (100 %)



Table 3- Table (3): Incidence of *E. coli* isolated from soft cheeses in El-Gharbia -Egypt :(EMB) :

Type Of Sample	Number of The Sample	Number of Isolate	Percentage of Isolation
Soft Cheese	150	64	42.5 %
Kariesh Cheese	50	27	54%
Tallage Cheese	50	16	32%
Domiatti Cheese	50	21	42%



Tables .3- 2. Regional Incidence of E-coli in Soft Cheese (EMB) :

Regions/Karish Cheese	Number	Positive Results	Percentage
Kafer EL-Zayate City	10	2	20 %
EL-Santta	10	Zero	Zero
EL-Mahlla EL- Cobra	10	10	100 %
Kottor	10	10	100%
Tanta	10	5	50 %



Regions/DomiattCheese	Number	Positive Results	Percentage
Kafer EL-Zayate City	10	Zero	Zero
EL-Santta	10	10	100 %
EL-Mahalla EL-Cobra	10	5	50%
Kottor	10	5	50 %
Tanta	10	1	10



Regions/ TallagaCheese	Number	Positive Results	Percentage
Kafer EL-Zayate City	10	ZERO	ZERO
EL-Santta	10	ZERO	ZERO
EL-Mahlla EL-Cobra	10	4	40%
Kottor	10	7	70 %
Tanta	10	5	50 %



Table (4) : Biochemicals Tests of *E. coli*:-

Bacteria			Biochemical tests
	Indole	Methyl red	Simmon citrate
<i>E. coli</i>	+	+	-



Table (5): Results of serological identification of *E.coli* isolates:- .

Serial	Identified Bacterium	Serodiagnosis	Strain
1	E.coli(K3)	O119 : H7	EPEC
2	E.coli(K1)	O111 : H8	EHEC
3	E.coli(K2)	O86	EPEC
4	E.coli(K5)	O18 :H7	ExPEC
5	E.coli(K6)	O128	EIEC
6	E.coli(D3)	O124	EPEC

K =Kariesh cheese , D=Domiatti cheese , T= Tallaga cheese .



Table (6):Antimicrobials sensitivity results for *E. coli* isolates according to Mohammed (2018).

S= sensitive

R= Resistant

I = intermediate

K= Kariesh cheese

D=Domiat cheese

Antimicrobial agent	Diffusion zone break point (mm)	D3 E.coli O124	E.coli K30119	K2 E.coli O186	K1 E.coli O111	E.coli K5018	Sensitivity Percent
Ciprofloxacin (CIP)	12 (mm)	S(20)	S(28)	S(29)	S(27)	S(32)	100%
Enrofloxacin (ENR)	0.5(mm)	S(8)	S(9)	R(0.3)	S(7)	S(8)	83.33%
Chloramphenicol (C)	15(mm)	S(23)	S(26)	R(7)	R(8)	I(17)	59%
Oxytetracycline (OT)	14(mm)	S(22)	S(21)	R(11)	S(25)	S(24)	83.33%
Neomycin (N)	12(mm)	I(14)	R(9)	R(8)	R(9)	R(7)	%^
Neofurazone (F)	32(mm)	R(25)	S(145)	R(24)	R(19)	S(149)	50%
Erythromycin (E)	13(mm)	R(9)	I(17)	R(9)	S(28)	R(7)	25%



Table (7): Results of molecular identification of *Stx1*, *eae A* and *fim H* genes of *E. coli* :-

Sample	eaeA	fimH	Stx1
1	-	+	-
2	-	+	-
3	+	+	-
4	+	+	-
5	-	+	-
6	-	+	-
7	-	+	-



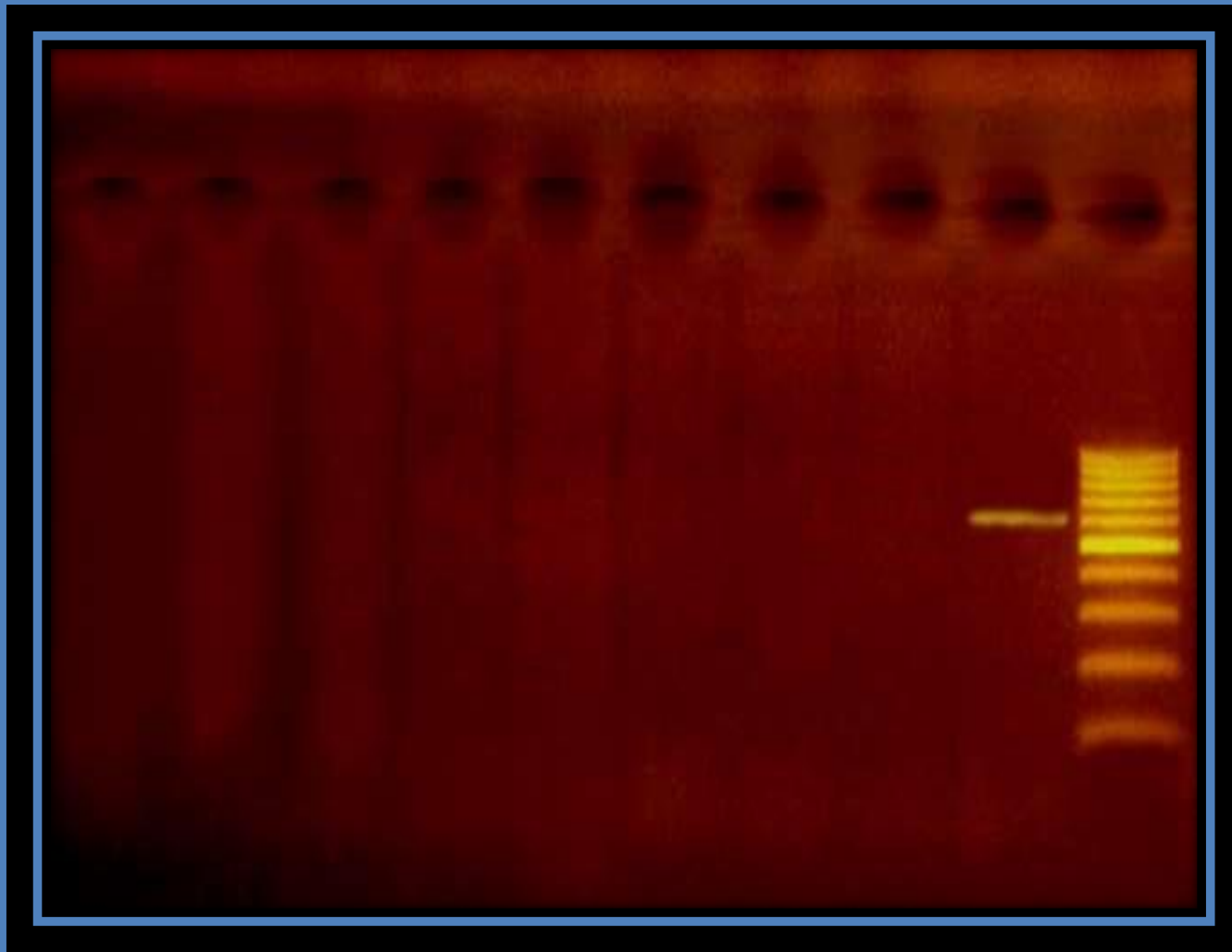


Figure (1): Agarose gel electrophoresis of PCR amplified products of virulence gene. For stx1 genes were negatives .



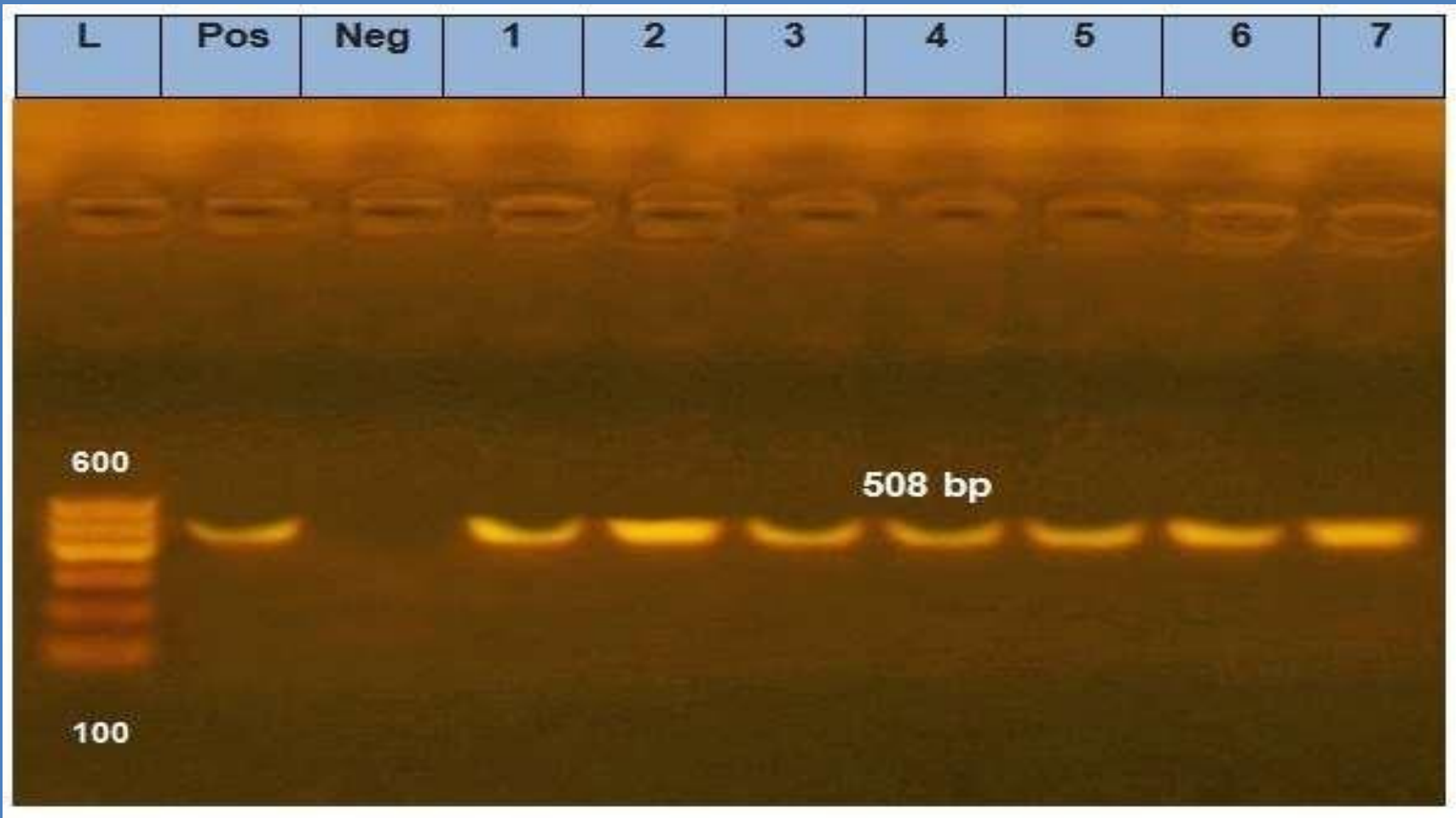


Figure (2): Agrose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Pos: Positive control, lane Neg: Negative control, Lane 1,2,3,4,5,6,7: *fimH* virulence gene of *E. coli*. The size in base pairs (508bP) of PCR product is indicated for the bands. Normal because it is a common in all *E. Coli*.



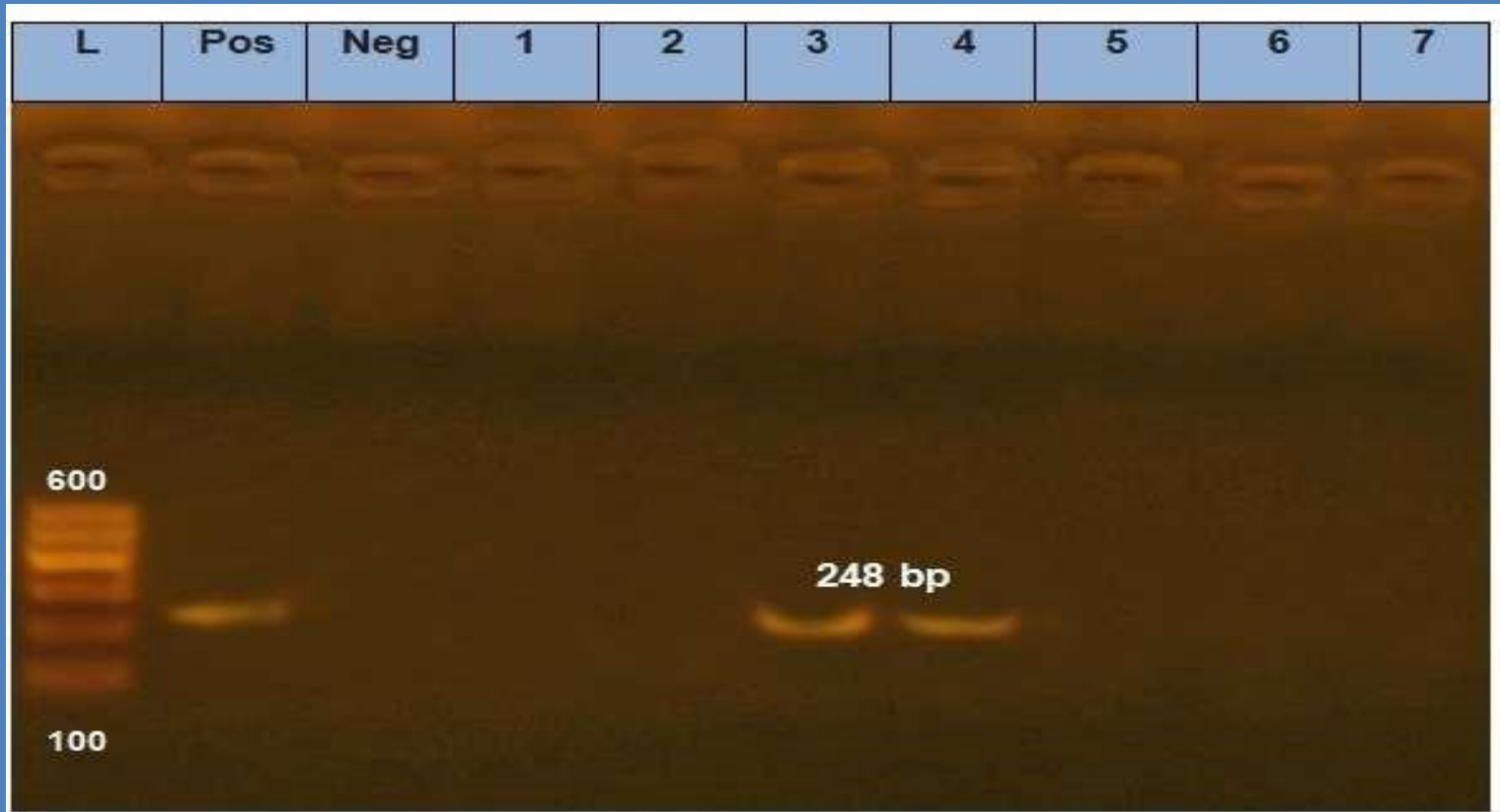


Figure (3): Agarose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Pos: Positive control, lane Neg: Negative control, Lane 2,3: *eaeA* virulence gene of *E. coli*. The size in base pairs (248bP) of PCR product is indicated for the bands. (It Is aco)





Discussion



5- Discussion

E.coli has largely been regarded as opportunistic pathogen causing of the intestinal and extraintestinal diseases associated with large numbers of food poisoning outbreaks (varnas and Evans, 1991).

Enteropathogenic *E.coli* causes about 30-40 % of infantile diarrhea, as it is the most frequently isolated bacterial pathogen from the cheeses (Doyle and Padyhe,1989).

Escherichia coli is commonly present in the intestinal tract of humans and animals, and its detection in processed foods is an indication of fecal matter contamination. It also indicates the inadequate control of the environment of the cheese processing unit, the personal hygiene of the food handlers, and the poor quality of the finished product (Kousta *et al.*, 2010; Dias *et al.*, 2012). Because the occurrence of an outbreak of a foodborne disease is caused by enteropathogenic *E. coli* and is associated with the consumption of soft ripened cheese (Marier *et al.*, 1973), the presence of these microorganisms in cheese has taken on added significance.



***E. coli* is responsible for several outbreaks of diarrhea in children and adults after ingestion of contaminated milk and dairy products. Different studies showed that 1 - 5% of food-borne infections were related to consumption of milk and dairy products, that 53% of cases of food-borne infections caused by contaminated cheese and that enteropathogenic *E. coli* (EPEC) is the causative agent of 18.33% of these cases (Schade *and* Yaeger, 2001).**



EPEC strains have been implicated in food-borne human illnesses, especially as an important agent of infantile diarrhea in developing countries (**Silva *et al.*, 2001**). EPEC strains are genetically mutative and cause different types of diarrhea in patients. A combination of these strains with enterohemorrhagic *E. coli* increases their ability to create systematic infections in humans (**Desilk *et al.*, 1991**).



The pathogenicity of EPEC strains is not known completely but most of them produce Vero toxins which are different from the enterotoxigenic *E. coli* (ETEC) toxins (**Campos *et al.*, 1994; Crossly *et al.*, 1955**). Some EPEC strains are attached to intestine epithelium (**Campos *et al.*, 1994**). Different strains of EPEC cause a different level of infection, and it has been shown that these strains are continuously changing and their genome becomes similar to hemorrhagic strains (**Campos *et al.*, 1994**).



Coliforms could be found in cheeses and are used as a hygienic indicator for such products. The presence of coliforms in cheese and their relation to enteropathogenic *E. coli* in soft cheeses has received considerable attention in previous studies (**Schade *and* Yeager, 2001; Maurier *et al.*, 1973**). The relation between pathogenicity and different serotypes of *E. coli* has been suggested and proved (**Crossly *et al.*, 1995**).



Contamination of dairy products by EPEC strains has been investigated and O126, O128, O25 and O125 were isolated (**Abba, 1988; Ahmed *et al.*, 1988**). Cheese has been known as an important animal origin product and in most countries domestic and traditional produced cheese are in demand.



Shiga toxin-producing *E. coli* (STEC) are among the most important causes of foodborne diseases. They are responsible for several human gastrointestinal diseases, including watery or bloody diarrhea (**Paton & Paton, 1998**). The common feature and the primary virulence factor of STEC is the production of Shiga toxin 1 (Stx1), Shiga toxin 2 (Stx2) or its variants, or a combination of these. Highly human-virulent STEC strains are also called enterohemorrhagic *E. coli* strains (**Bolton, 2011**).



Food is an important vehicle for the transmission of STEC strains, and most of the foodborne outbreaks caused by STEC have been associated with unpasteurized milk and its derivatives (Farrokh *et al.*, 2013). The aim of this study was to assess the STEC occurrence in the collected cheeses samples.



essential for the intimate attachment and formation of attaching and effacing lesions on gastrointestinal epithelial cells (**Bolton, 2011**). Most outbreaks and sporadic cases of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) have been attributed to strains of serotype O157:H7. However, in some countries, the importance of non-O157 STEC as causes of HUS, HC, and other gastrointestinal disease is being increasingly recognized, particularly the serogroups O26, O103, O111, O121, O45, O145, and O91 (**Farrokh *et al.*, 2013**).

Illness caused by enterohaemorrhagic E.coli can range from self limited watery diarrhea to life threatening manifestations such



Recently there is additional concern about the presence of E.coli in cheeses because of the ability of some strains to cause food borne illness and lead to high incidence of food outbreaks (**chapman, 2000**).

As milk is of high nutritive value containing vitamins, minerals and proteins so it promotes the growth of E. coli and another many microorganisms as a result of contamination from using unsterilized utensils , improper preparation of dairy animals, uses of contaminated water supplies or careless method of handling (**Trevena *et al.*, 1999**).



Kariesh cheese is considered as one of excellent sources of protein, phosphorus, calcium and many trace elements and due to its high nutritive value and low prices it is considered as a popular food in Egypt. It is manufactured from skim milk naturally fermented by lactic acid bacteria at home , so it may be contaminated from sources during its manufacture, handling and distribution that renders it a source of infection with many diseases **(Thabet, 2003)**. Therefore, evaluation of hygienic quality of kariesh cheese manufactured in el Gharbia governorate is one of the objects of this study.



coli, inactivation of *E. coli* shiga toxins has not been proven.

However, following consumption of pasteurized milk, an outbreak in North Cumbria, England, during 1999 with HUS cases have been reported and no living bacteria were found in the milk samples (**Goh *et al.*, 2002**).

Epidemiological studies suggest that nonO157 Shiga toxin-producing *E.coli* (STEC) is a major player associated with foodborne disease outbreaks (**Johnson *et al.*, 2006**).

The ten most clinically relevant STECs belong to serogroups O26, O103, O111, O145, O157, O91, O113, O128, O45, and O121 have been reported in milk and dairy products causing severe illness in humans including diarrhea, hemorrhagic colitis, and hemolytic



growth due to synergistic effect of different factors exist in cheese as (low pH, water activity, NaCl and starter culture) (**Galvez *et al.*, 2007**) .

In this work the detection of *E.coli* in soft cheeses was performed by isolation and identification of enteropathogenic *E.coli* strains serotypes.

In the present study a total number of 150 samples of kariesh cheese, tallaga cheese, domiatte cheese (50 samples of each) were collected from different localities in el – Gharbia governorate, Egypt and examined for the presence of E-coli. The tallaga and domiatte cheese from cheese dairy shops and kariesh cheese from both dairy shops and street vendors



cheese proved by selective culturing on (Eosin Methylene blue media) were isolated and identified as E –coli as fellow: Table (3)

1- Kariesh cheese:

Inspection of table before revealed that *E.coli* could be isolated from examined Karees cheese samples with incidence rate of 54%. Nearly similar finding was reported by El-bajory *et al.*, (2015), El- Nahass *et al.*, (2015), Nigh *et al.*, (2012). Higher incidence was reported by Ibrahim *et al.*, (2019), Embark *et al.*, (2016),El-kosi (2001),Mohammed (2015) while lower incidence was reported by El-Sayed *et al.*, (2011), Zeinhom (2011), Hosni *et al.*, (2011), Hefley(2012), Abd EL- Latif, eman (2012), Ahmed (2012), Brooks *et al.*, (2012), Nagah *et al.*, (2012), Samaha *et al.*, (2012), Elhadidy and Mohammed (2013), Bunyanian *et al.*, (2014), Abo Zed (2014), Mutually and Fatma (2015), El Nahass *et al.*, (2015), Gamal *et al.*, (2 015), Jaehn *et al.* (2015) Gamal and Soad (2016) Sameha awed (2016) and Lotfy *et al.*

2-Damietta Cheese.

It is evident from the table before that *E.coli* could be isolated from examined Damietta cheese samples with incidence rate of 42%. Nearly similar finding was reported by Ozen *et al.*, (2011), Ibrahim *et al.*, (2019) while lower incidence was reported by Zeinhom (2011), Hefney (2012), Nagah *et al.*, (2012), Elhadidy and Mohammed (2013), Gamal and Soad (2016), Jehan *et al.*, (2015) ,Gamal *et al.*, (2015), Elbassary (2006) , El-bajory *et al.*, (2015) and Lotfy *et al.*



3-Tallaga cheese: It is evident from the table before that *E. coli* could be isolated from examined tallaga cheese samples with incidence rate of 42%. Nearly similar finding was reported by **El-Sayed *et al.*, (2011)**, while lower incidence was reported by, **Hefney (2012)**, Contamination of milk and milk products, with *E. coli* is largely due to unhygienic condition during production, processing, handling, and distribution. *E.coli* is a good indicator of fecal pollution as it exists in the normal microflora of the intestinal tract of humans and warm blooded animals (**Olfa *et al.*, 2013**).



and even causes outbreaks and death of the consumers because cheeses eating without further heating for pasteurizations.

In this study *E coli* was isolated from 43% of samples, and 75% of isolates belonged to the EPEC serogroup. Recent studies in other countries indicate similar results. **Araujo *et al.*, (2002)** detected high levels of fecal contamination in 95.5% of cheese samples in Brazil, and EPEC was isolated from 21.1% of the samples. In Egypt, 60 Karish cheese samples were examined and *E. coli* was isolated from 75% of the samples. Similarly, **Abbar and Kadder (1991)** reported that 40.5% of cheese samples in Iraq were contaminated with EPEC strains..In the present study we detected 6 different EPEC serogroups in Egyptian soft cheese and that O127 was not found serogroup. Recent studies in other parts of world indicate different prevalent EPEC serogroups in soft cheeses. In Brazil, O127 was the most frequently found serogroup, followed by O55 and O26 (**Araujo *et al.*, 2002**). **Ahmed *et al.*, (1988)** showed that most prevalent serogroups in Egypt are O111, O126, O128, O26, O25 and O125. In Iraq, four EPEC serogroups, including O111, O86, O125 and O119, are commonly isolated from cheese samples (**Abbar, 1988**). In this study, EPEC serogroups were detected as O119 serogroup. Similarly to our results, **Abbar(1988)** reported that O119 is one of the prevalent EPEC serogroups in cheese. In Australia, O119 serogroup was isolated from 161 patients with



In another study in the west of Iran, *E. coli* was isolated from 51% of domestic Iranian soft cheese samples (**Shidfar *et al.*, 2004**). It appears that the rate of contamination of soft cheese with *E. coli* varies in different parts of Iran.

According to the results of this study, 19.48% of isolated *E. coli* strains from domestic soft cheese belong to EPEC strains.

3-Sensitivity test : Table (6)

The best antibiotics for treatment of colibacillosis infections in animals and humans were : Ciprofloxacin , Enrofloxacin , Oxytetracycline respectively to avoid the antimicrobial resistance . The presence of antimicrobial resistance with virulence genes among STEC strains is worrisome. Therefore, when relationship between virulence genes and antimicrobial resistance occurs the antimicrobial resistance might be selected through the antimicrobial use, and as a result, the virulence genes would be selected as well.



It is evident from the table before that *E.coli* virulence genes or pathogenicity that causes public hazard on cheeses consumers even cause could be discussed as follows :Table (2,7) :

1. *Stx1* gene It is shiga toxin potent verocytotoxic and cause sever illness :

Its prescence in our isolates by PCR was zero % and not found in all *E.coli* isolates Nearly similar findings was reported by **Taha *et al.*, (2019)** while higher incidences findings were reported by **Ibrahim *et al.*.,(2019)**, **Sabrey *et al.*, (2008)**, **Hussien *et al.*, (2019)**, **El-hadidy and Mohamed (2012)**, **Ombark *et al.*, (2016)**, **Chalechtori *et al.*, (2017)**.

1. *Eae A* gene it an intimin secretion gene that enhance attachment and colonizations :

Its prescence in our isolates by PCR was 28.5 % of *E.coli isolates* : while lower incidences findings were reported by **Ibrahim *et al.*, (2019)**, **Sabrey *et al.*, (2008)**, **Hussien *et al.*, (2019)**, **El-hadidy and Mohamed (2012)**, **Chalechtori *et al.*, (2017)**.



fliC_H gene. It is responsible for biofilm formation and enhance infection of Uropathogenic *Escherichia coli* and the structural role of type 1 fimbriae and responsible of about 92 % of the pathogenicity and diseased cases in human patients suffering from Urinary tract infections :

Its presence in our isolates by PCR was 100 % of *E. coli* isolates : similar results and findings were reported by **Garofalo *et al.*, (2007)** while lower incidences findings were reported by , **Watts *et al.*, (2010)**, **Mladin *et al.*, (2009)**, **Arabi *et al.*, (2012)**, **Tarchouna *et al.*, (2013)**, **Sabrey *et al.*, (2008)**, , **Zohreh *et al.*, (2014)**, **Ibrahim *et al.*, (2019)**.

Current study supports the finding that raw milk and various milk products can be regarded as critical source of : pathogenic *E. coli* This explains the need of strict monitoring and surveillance for effective measures of hygiene and sanitary practice during production of milk and various milk products .

According to **Egyptian standard (2005)** which stipulated that milk and milk





Conclusion and Recomendations



source of pathogenic *E. coli* . This explains the need of strict monitoring and surveillance for effective measures of hygiene and sanitary practices during production of soft cheeses (kariesh, domiatti , tallaga) . The obtained results in this work give an information that some of examined soft cheese like Kareish cheese, Damietta cheese and Tallaga cheese samples sold in Gharbia governorate were of poor microbiological quality and that indicate inadequate hygienic measures adopted during their production, handling, processing, packaging and distribution of the examined soft cheeses products.



Contamination of the examined Kareish cheese, Damietta cheese and Tallaga cheese samples with pathogenic microorganism with *E.coli* is a reliable index of fecal pollution, poor personal hygiene, constitute a public health hazard can range from self-limited watery diarrhea to life threatening manifestations such hemorrhagic colitis, hemolytic uremic syndrome, thrombocytopenic purpura and may lead to death and may lead to food poisoning and undesirable changes of the



Therefore, to safe guard consumers from being infected and to improve the quality of Kareish cheese, Damietta cheese and Tallaga cheese products the following suggestions should be taken in consideration:

6.1-General recommendations to minimize soft cheese contamination:

- Good quality clean milk is essential for production of cheese.
- The milk should be pasteurized undergo a full pasteurization or an equivalent process to avoid hazards from pathogens.
- Raw milk should be stored at 2° C before processing into cheese.
- Cleaning equipments by scrubbing with brush and warm detergent then rinse with cold water and sterilize with boiling water.
- Good hygienic practices during cheese manufacture is very important



production and handling of soft cheeses should provided.

- Only healthy persons who have a sense of hygiene should be employed and they should pass a periodical medical examination.
- Adequate control through periodical examination inspections of shops and small cheeses factories by specialists to insure those errors are corrected.
- HACCP system should be applied which is scientific and systematic identifies a specific hazard throughout the food chain, from primary production of milk and cheese till it reaches the consumers. The most important reason for adopting HACCP approach is that no other quality





In conclusion, it deems necessary that concerned authorities should impose regulations and bacteriological standards for control of soft cheeses production and handling not only for detection of errors and defects but to insure that the errors are corrected and defects are not repeated and ensure a maximum of safety to the consumers and stipulated to prevents any cheeses without excellent packaging's and labelled stamped details of recent quality control measurements of factory and manufacturers to easy detections and executions the laws of health authorities to prevents corruptions and health outbreaks(preventing homes manufactures) . communications .

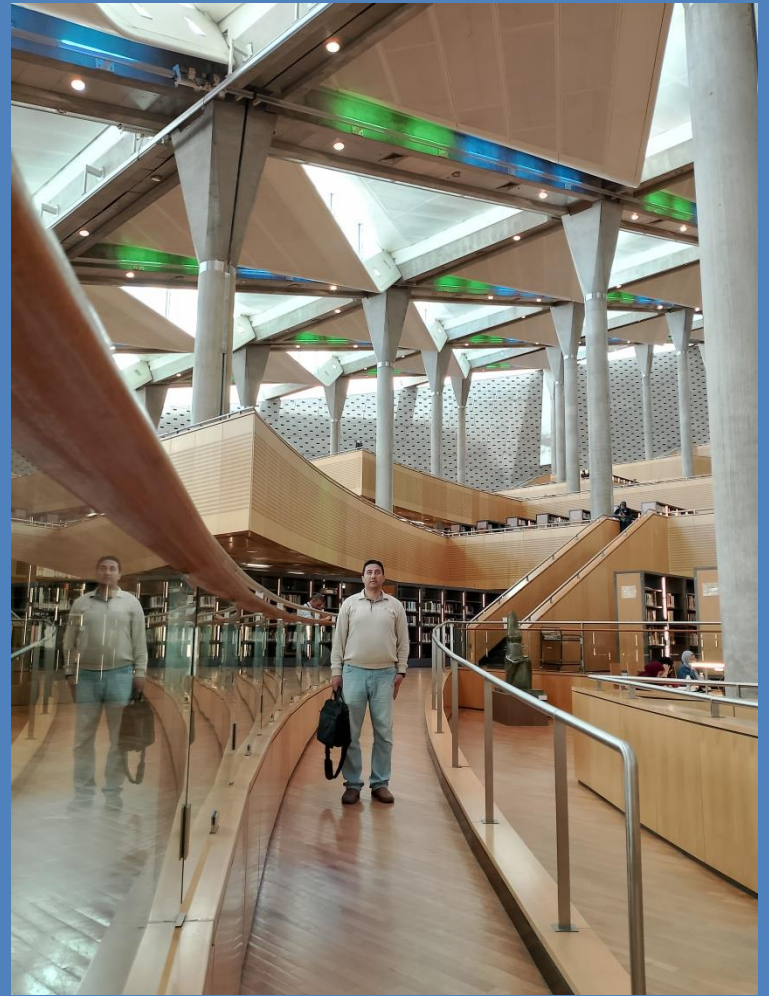




The previous sot types of cheeses are very important as asource of high quality essential fatty acids for nervous systems stabilizations and communications and also essential fat soluble vitamins likes A ,K,D.E. (Kariesh , Domiatti and Tallaga) but under complete health authority laws.



Summary



7- Summary

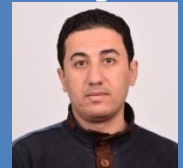
One hundred and fifty samples, 50 each of Kareish cheese, Damietta cheese and Tallga cheese were collected from supermarkets, dairy shops and street vendors in El- Gharbia governorate for microbiological analysis.



Microbiological examination of the examined samples revealed that *E. coli* could be detected in 54%, 42% and 32% of examined Kareish cheese, Damietta cheese and Tallage cheese samples, respectively. Serological typing of isolated strains of *E. coli* proved that they belong to six serotypes: O1119, O111, O86, O18, O128, and O124. It is presences in cheeses as responsible as orders indicates dust - air- spitting – sneezing- dried urinations – dried feaces – fresh urinations- infected workers hands with one of all the above singly or together .The serologically identified pathotypes of *E.coli* isolates from some types of soft cheese were :



were : ((O119) (EPEC) Enteropathogenic *Escherichia coli*, (111) (EHEC) Enterohemorrhagic *Escherichia coli*, (O86) (EPEC) Enteropathogenic *Escherichia coli*, (O 18) (Ex PEC) Extera intestinal pathogenic *Escherichia coli*, (O128) (Atypical EPEC) Atypical EPEC *Escherichia coli*, (O124) (EIEC) Entero Invasive *Escherichia coli*. *E. coli* infections from contaminated cheeses surly constitutes publics hazards of living creatures especially in humans likes meningoencephalitis, pyelonephritis, hepatitis, pneumonitis, pleurisy, pericarditis, peritonitis, osteomyelitis a appendicitis.



And even causes outbreaks and death of the consumers because cheeses eating without further heating for pasteurizations.

In conclusion, isolation of EPEC serogroups from domestic soft cheese represents a potential, as well as an indication, of the presence of other enteropathogenesis. Although recent studies on virulence factors indicate that not all EPEC strains are able to attaching effacing lesion, it is however believed that high prevalence of contamination with EPEC strains increases the risk of infection for children, due to consumption of domestic soft cheese. It seems that further epidemiological investigations are needed to reveal the

importance of contamination in domestic soft cheese in this area of Egypt.

العربي الملخص

من خلال الفحص المناعي النوعي المعملی التخصصی وجد أن هنالك أربع معزولات من بكتيريا الإشيريشية لقولونية المعوية الممرضة بذاتها والتي تعتمد على قدرتها في تدمير الخلايا وتشوهها مما ينتج عنه تدميرها وإفراغ محتواها البيولوجي وتكاثر ونمو البكتيريا على المغذيات الخلوية وسببت العديد من النكسات الصحية في العالم كله خصوصا في أمريكا وأوروبا سنة ١٩٨٢ وسنة ١٩٨٧ على التوالي وهم كالتالي :



O119,O86,O18,O128

وبالتالي يجب الحذر منها لأنها قد تسبب في إهلاك الآلاف من الإسهال الشديد والجفاف بالرغم من العناية في المستشفيات وأخذ المضادات الحيوية فوصلت عدد الإصابات إلى سبعة وعشرون ألف إصابة وموت أكثر من ألفين في أمريكا في إحدى ولاياتها فقط وهكذا نفس العدد في قارة أوروبا وهؤلاء خمس معزولات من الجبن القريش المباعة في مصر محافظة الغربية ومعزول واحد من الجبن لدماطي وهو ينتمي إلى البكتيريا الإشيريشية قوية الإختراق سريعة الحركة وهي مدمرة للخلايا وسبب لنكسات صحية والإسهال الدموي والبولي ويمكن أن يسبب الفشل الكلوي والمخى والرئوى والموت لا قدر الله وهو

O124





وأیضا تم التأكد من سبع معزولات من الجبن الطرية المذكور أنواعها بإنزیم البلمرة المتسلسل وجميعهم يحتوى على جین التجمع الخلوى البكتيري المختص بخلايا الجهاز البولى والتناسلى فى البشر والحيوانات وتسمى البكتيريا الإيشيريشية البولية ويمكن أن يسبب الفشل الكلوى وتقرح المثانة وإلتهابات مزمنة بالجهاز البولى والتناسلى ومن المهم أيضا معرفة أحسن المضادات الحيوية لعلاجها والقضاء عليها وهى بالترتيب بالتأكد المعملی : السيبروفلوكساسين والإنزوفلوكساسين والأوكسى تترسيكلين منعا للنكسات الصحية والعجز الخلوى والفشل الكلوى والكبدى والمخى ويمكن لا قدر الله وبدون أعراض حتى لا يوجد إرتفاع فى درجة الحرارة . ومعزول واحد من الجبن القريش ويسمى البكتيريا الإيشيريشية القولونية النزفية وهى تفرز سم الهيموليسين المدمى للخلايا والأوعية الدموية وتكسر الخلايا الدموية المسماة بكرات الدم الحمراء وتعرفها بوجود صبغة الهيموجلوبين فى البراز والبول وتسبب الظاهرة المرضية المعروفة عالميا التحلل الدموى الكلوى وتسبب الوفاة ومقاومة لكل المضادات الحيوية علما بأن الجبن تؤكل بدون معاملة حرارية وما يحدث فى الكلى يحدث فى باقى الأعضاء الحيوية وهو



ومن خلال دراساتي وإستبياناتي العلمية العملية تأكدت من الجبن المباعه بدون مراقبة وفي المنازل كبعض أنواع الجبن القريش والجبن الدمياطى والجبن التلاجة وبدون تغليب وبيانات واضحة على العلب توضح أنها لم تخضع لمراقبة قوانين الجودة المصرية وأنها غير مطابقة للمواصفات القياسية حسب الشروط والإلزامات الصحية التى صدرت من السلطات المختصة بإنتاج جبن طرية عالية الجودة وخالية من مسببات الأمراض نهائية مهما كانت بكتيرية وفطرية وفيرروسية وعليها بيانات واضحة بإسم المصدر أو المصنع أو المحل ويكون مرخصا وعليه بيانات الإتصال واضحة لضمان الشفافية والمراقبة وعدم إختلاط الأمور على السلطات فى إنفاذ القوانين ومنع الفساد أو إنتشار الأمراض خصوصا المميته منها فى صغار وكبار السن ومرضى نقصان المناعة والإلتهابات المزمنة التى تؤدى إلى الفشل العام الوظيفى ويؤدى لا قدر الله إلى الوفاة بأعداد عديدة و إنهاك الدولة إقتصاديا وصحيا بدون داعى .





يجب الحذر من إستخدام الإنروفلوكساسين والسيبروفلوكساسين فى علاج الطيور بشكل عام وخصوصا الكتاكيت التى تعاني من كل أنواع الإسهالات مهما كان لونه أبيض أو أخضر أو أصفر أو دموى لأن هنالك مناعة دوائية خلقية وليست خولقية فمهما تعطيها من جرعات عالية فلن يؤثر على علاجها ولكن سيسبب القتل الكيميائى لهن ويؤدى إلى الفشل الكلوى والكبدى والمخى لكى يقتل البكتيريا منها الإي كولاى الشائعة فى الإصابة المعوية فى صغار ذوات الدم الحار وطبعا البارد ولكنها أقل خطورة لأنها ستكون تسمم دموى خفيف يعيق بعض نموها وبالتالي سنلجأ إلى الأوكسى تتراسيكلين لأن الكلورامفينكول قد تم تحريمه دوليا ويسبب أمراض خطيرة على مستهليكيها من البشر ولهن وإذا كان يسبب بعض الأعراض فهو يؤخذ بشكل عام جرعة واحدة فقط وتكرر ساعة اللزوم وله تأثير قاتل على طفيليات الدم بشكل عام مثل البابيزيا والثيليريا والأنابلازما والتربانوسوما امراض التيك أو لعنة الله على الفراعنة وهنالك من يقول أن الأوليان يسببان هشاشة العظام كلام المعتقدون لان يجب إستخدامه بشكل مطول لأشهر هذا لا يحدث أبدا إلا إذا كان هنالك مقاومة دوائية فليس العيب فى الدواء ولكن فى التشخيص .

